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Development of an Enzyme-Linked Immunosorbent Assay to Detect Chicken Parvovirus-Specific Antibodies

Keith O. Strother and Laszlo Zsak[A]

Southeast Poultry Research Laboratory, U.S. Department of Agriculture, Agricultural Research Service, 934 College Station Road, Athens, GA 30605

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SUMMARY. Here we report the development and application of an enzyme-linked immunosorbent assay (ELISA) to detect parvovirus-specific antibodies in chicken sera. We used an approach previously described for other parvoviruses to clone and express viral structural proteins in insect cells from recombinant baculovirus vectors. In baculovirus recombinant-infected Sf9 cells, the chicken parvovirus (ChPV) structural viral protein 2 (VP2) was detected as an abundant protein, and the 60-kDa VP2 strongly reacted with parvovirus-infected chicken serum in Western blot. A semipurified VP2 was then used in capture ELISA. Sera from chickens experimentally infected with ChPV and sera from uninfected chickens were tested to evaluate the assay. The ELISA was 93.3% sensitive and 100% specific in detecting ChPV-infected birds. Subsequent assays identified IgG type ChPV-specific maternally acquired antibodies in day-old chickens and demonstrated the production of virus-specific antibodies in young birds following infection with ChPV. In our study, a specific antibody response of infected chickens was observed starting with IgM production between 14 and 21 days postinfection (DPI) and switching into a predominant IgG response by 32 DPI. The availability of an ELISA for detection of virus-specific antibodies and its ability to differentiate between maternally acquired antibodies and antibodies produced following acute infection could prove to be a valuable tool to characterize pathobiological properties and immunogenicity of ChPV.

RESUMEN. Desarrollo de un ensayo de inmunoabsorción con enzimas ligadas para detectar anticuerpos específicos contra parvovirus de pollo.

En este trabajo se reporta el desarrollo y aplicación de un ensayo de inmunoabsorción con enzimas ligadas (ELISA) para detectar anticuerpos específicos de parvovirus en sueros de pollos. Se utilizó un método descrito previamente con otros parvovirus para clonar y expresar las proteínas estructurales virales en células de insecto utilizando baculovirus recombinantes. En las células Sf9 infectadas con baculovirus recombinantes, se detectó de manera abundante la proteína estructural VP2 de parvovirus de pollo (ChPV). Esta proteína de 60 kDa reaccionó fuertemente con suero de pollos infectados con parvovirus mediante la técnica de inmunoelectrotransferencia. La proteína VP2 semipurificada fue utilizada en una ELISA de captura. Sueros de pollos infectados experimentalmente con ChPV y sueros de pollos no infectados se utilizaron para el evaluar el ensayo. La prueba de ELISA mostró una sensibilidad de 93.3% y una especificidad de 100% para detectar aves infectadas con ChPV. Ensayos subsecuentes identificaron anticuerpos de origen materno tipo IgG específicos contra ChPV y también demostraron la producción de anticuerpos específicos contra el virus en aves jóvenes después de la infección con ChPV. En este estudio, se observó que la respuesta por anticuerpos específica en aves infectadas iniciaba con la producción de IgM entre los días 14 y 21 después de la infección y cambiaba a una respuesta predominantemente por IgG por el día 32 después del desafío. La disponibilidad de un ensayo de ELISA para la detección de anticuerpos específicos contra el virus y su capacidad para diferenciar entre anticuerpos de origen maternal y los producidos después de una infección aguda, demostraron ser de utilidad para caracterizar las propiedades patobiológicas y la inmunogenicidad del ChPV.

Key words: parvovirus, ELISA, chicken, poultry, enteric disease

Abbreviations: BV = baculovirus; ChPV = chicken parvovirus; CV = coefficient of variation; DPI = days postinfection; ELISA = enzyme-linked immunosorbent assay; NS = nonstructural gene; PAGE = polyacrylamide gel electrophoresis; PCR = polymerase chain reaction; PEC = poult enteritis complex; PEMS = poult enteritis mortality syndrome; RLU = relative light unit; RSS = runting-stunting syndrome; RT = room temperature; SDS = sodium dodecyl sulfate; SEPRL = Southeast Poultry Research Laboratory; SPF = specific pathogen free; TE = Tris-ethylenediaminetetraacetic acid; VP = structural viral protein; WB = washing buffer

Viral enteric disease of poultry is a significant economic problem worldwide. The two major enteric disease forms are poult enteritis complex (PEC) in turkeys and runting-stunting syndrome (RSS) in broiler chickens (3,9,22,23,26,28,32). Poult enteritis mortality syndrome (PEMS) is an enteric disease of turkeys that is characterized by high mortality rates (2).

The etiologic agents of poultry viral enteric diseases include many potential candidates such as reovirus, coronavirus, rotavirus, astrovirus, and enterovirus (6,7,11,17,23,24,25,26,27,29,31,32,34). Currently, the role of some of these viruses in the etiology of enteric diseases is not completely understood. A vaccine for PEC, PEMS, or RSS has not yet been developed because of the ambiguity concerning the exact etiologic agents of these diseases. Management of poultry enteric diseases includes cleaning and disinfection of poultry houses, increased biosecurity, and antimicrobial therapy to reduce the effect of bacterial coinfection (2,3).

In earlier studies, parvoviruses have also been identified in turkeys and chickens exhibiting enteric disease (16,33,36). Inoculation of day-old broiler chickens with purified parvovirus particles resulted in typical clinical signs of RSS (14). However, these parvoviruses have not been characterized using more definitive methodologies, and their role in the etiology of enteric disease has not been further studied.

Recently, we described a molecular screening technique for the detection of novel viruses in clinical samples of chickens and turkeys exhibiting characteristic signs of enteric disease (36). The technique

[A]Corresponding author. E-mail: laszlo.zsak@ars.usda.gov

is based on random amplification of particle-associated nucleic acids in clinical samples. Using this method, we successfully identified parvovirus DNA sequences in intestinal homogenates from affected birds. Sequence analysis of the nonstructural gene (NS) of these novel parvovirus genomes demonstrated that the chicken and turkey parvoviruses represent an independent group within the *Parvoviridae* family (36).

Our recently developed polymerase chain reaction (PCR) assay with primers targeting the conserved NS genomic sequences proved to be highly specific and sensitive for detecting parvoviruses in experimentally infected chickens (35). A nationwide survey revealed that chicken and turkey parvoviruses are widely distributed in commercial poultry flocks in the United States. Although our data suggest that parvovirus infection is highly prevalent in poultry flocks affected by enteric disease, there was no definite correlation between virus presence and disease (35).

It has been well documented with other parvovirus infections, including goose parvovirus and human boca parvoviruses, that parvoviruses or their DNA can be frequently detected from both healthy and diseased individuals, especially at an early age of life (10,13). Importantly, as was shown in goose parvovirus infection, maternally acquired virus-specific antibodies play a significant role in the epidemiology of clinical disease, and the level of passive immunity determines the susceptibility of the progeny following virus infection (10).

In order to define the exact role of parvoviruses in the etiology of enteric diseases of poultry, a complex seroepidemiological study will be necessary. To study the epidemiology and biology of chicken and turkey parvoviruses, production of reagents and development of sensitive serologic assays became urgently important. Here, we report the development and application of a capture enzyme-linked immunosorbent assay (ELISA) to detect parvovirus-specific antibodies in chicken serum samples. We used an approach previously described for other parvoviruses to clone and express viral structural proteins in insect cells from recombinant baculovirus vectors (13,15,18,20). Baculovirus-expressed structural viral protein 2 (VP2) was then employed as antigen to develop an ELISA for detection of immunoglobulins directed against structural determinants in the viral particle. We have used this ELISA to demonstrate the presence of maternally derived parvovirus-specific antibodies in chicken serum samples and virus-specific antibodies in sera following experimental infection of birds.

MATERIALS AND METHODS

Virus. The ABU chicken parvovirus (ChPV) was isolated in Hungary in 1984 (16). A homogenate of intestinal tissue from chickens inoculated with the cesium chloride purified ABU virus (14) was kindly provided by J. Kisary. The homogenate was used to prepare ABU-P1 virus inoculum, as previously described (14). Briefly, 1-day-old specific-pathogen-free (SPF) white rock broiler chickens ($n = 10$) were received from the Southeast Poultry Research Laboratory (SEPRL) flocks and inoculated orally with the ABU virus homogenate. Seven days later, the chickens were killed and the entire intestinal portion was used to prepare a 10% homogenate in phosphate-buffered saline. The homogenate was clarified by centrifugation at 775 × *g*, and the supernatant containing the ABU-P1 virus was used as inoculum in further studies.

Chickens. SPF white rock chickens were obtained from the SEPRL flocks at 2 days of age and divided into three groups, with five birds in one group and 15 birds in each of two other groups. Among other agents, these flocks are monitored for avian reovirus, astrovirus, adenovirus, infectious bursal disease virus, and chicken anemia virus (32). Fast Cornish broiler chickens were received from a commercial hatchery at 2 days of age via FedEx transportation. Chickens were housed in Horsfal isolators (Federal Designs, Inc., Comer, GA) with *ad libitum* access to feed and water. General care was provided as required by the Institutional Animal Care and Use Committee, as outlined in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (5).

Table 1. Experimental infection of chickens.

Breed	Group (*n*)	ChPV[A] infection (age in days)	Age at bleeding (days)
White rock	A (5)	None	35
	B (15)	ABU-P1 (2)	37
	C (15)	None	37
Fast Cornish	FC-A (42)	None	2
	FC-B (38)	None	23
	FC-C (42)	ABU-P1 (2)	5, 14, 21, 32 DPI[B]

[A]ChPV = chicken parvovirus.
[B]DPI = days postinfection.

Experimental infection of chickens with ABU parvovirus. The experimental design for chicken studies is shown in Table 1. One group of five SPF white rock chickens (group A) was kept in an isolator for 33 days without inoculation as negative control birds. The birds were bled from the ulnar (wing) vein at the end of the experiment using microtainer serum collectors (Becton Dickinson, Franklin Lakes, NJ), serum was centrifuged at 3000 × *g* for 15 min, and the supernatants were kept at −20 C. A group of 15 SPF white rock chickens (group B) was inoculated at 2 days of age with 0.1 ml of the chicken parvovirus ABU-P1 strain by oral routes. The other group of 15 birds (group C) was not inoculated and used for direct comparison to group B birds. Serum samples were collected at 35 days postinfection (DPI) from each bird from the ulnar vein and processed as described above.

Two-day-old fast Cornish broilers were divided into three groups (FC-A, FC-B, and FC-C). Birds from the FC-A group ($n = 42$) were terminally bled for serum collection immediately, while FC-B birds ($n = 38$) were kept as uninoculated controls and bled for serum at 23 days of age. Forty-two chickens in FC-C group received 0.1 ml of the chicken parvovirus ABU-P1 strain by oral routes, and serum samples were collected at 5, 14, 21, and 32 DPI.

Construction of baculovirus recombinants. ABU-P1 virus supernatant from chicken enteric homogenate was used as a template for PCR to amplify the parvovirus structural genes VP1 and VP2. The primers PVBacF1 (5′-CACCATGAAATACAAAGCTCCGAAAGGC-3′) and PVBacR (5′-TTAGTTGGTCCGCGGCGCGCGCTTGGT-3′) for VP1 and PVBacF2 (5′-CACCATGATGGCAGATGAAAATGAA CCTG-3′) and PVBacR for VP2 were based on sequence data from our laboratory (Zsak *et al.*, unpubl. data). The VP1 gene was composed of 675 amino acids, while VP2, which is nested within VP1, had 536 amino acids (Fig. 1A). The BaculoDirect baculovirus expression system (Invitrogen, Carlsbad, CA) was then used to create baculovirus recombinants containing the two genes. Briefly, PCR-amplified VP1 and VP2 were gel purified and ligated into the entry vector pENTR and then transformed into *E. coli* (One Shot MaxEfficiency, Invitrogen) to create pENTR-VP1 and pENTR-VP2. Clones were tested for the presence of the inserted genes by PCR and restriction enzyme digests and subsequently sequenced to confirm the integrity of the gene. Finally, homologous recombination was performed between the pENTR vector containing the VP1 and VP2 genes and linearized baculovirus DNA to produce the recombinants ChPV-VP1 and ChPV-VP2. As a control, similar procedures were followed for insertion of the attachment protein G of avian metapneumovirus subtype C (21) (GeneBank accession number AY579780.1) into the baculovirus (BV) to create BV-aMPV(C)-G. In addition, a baculovirus not containing an insert was created by performing the homologous recombination reaction without the addition of an entry vector, which results in background self-ligation of the linearized baculovirus to create a circularized vector (BV only).

SF9 insect cells (Invitrogen) were next transfected with the baculovirus recombinants. A plaque assay was performed to isolate clones, which were

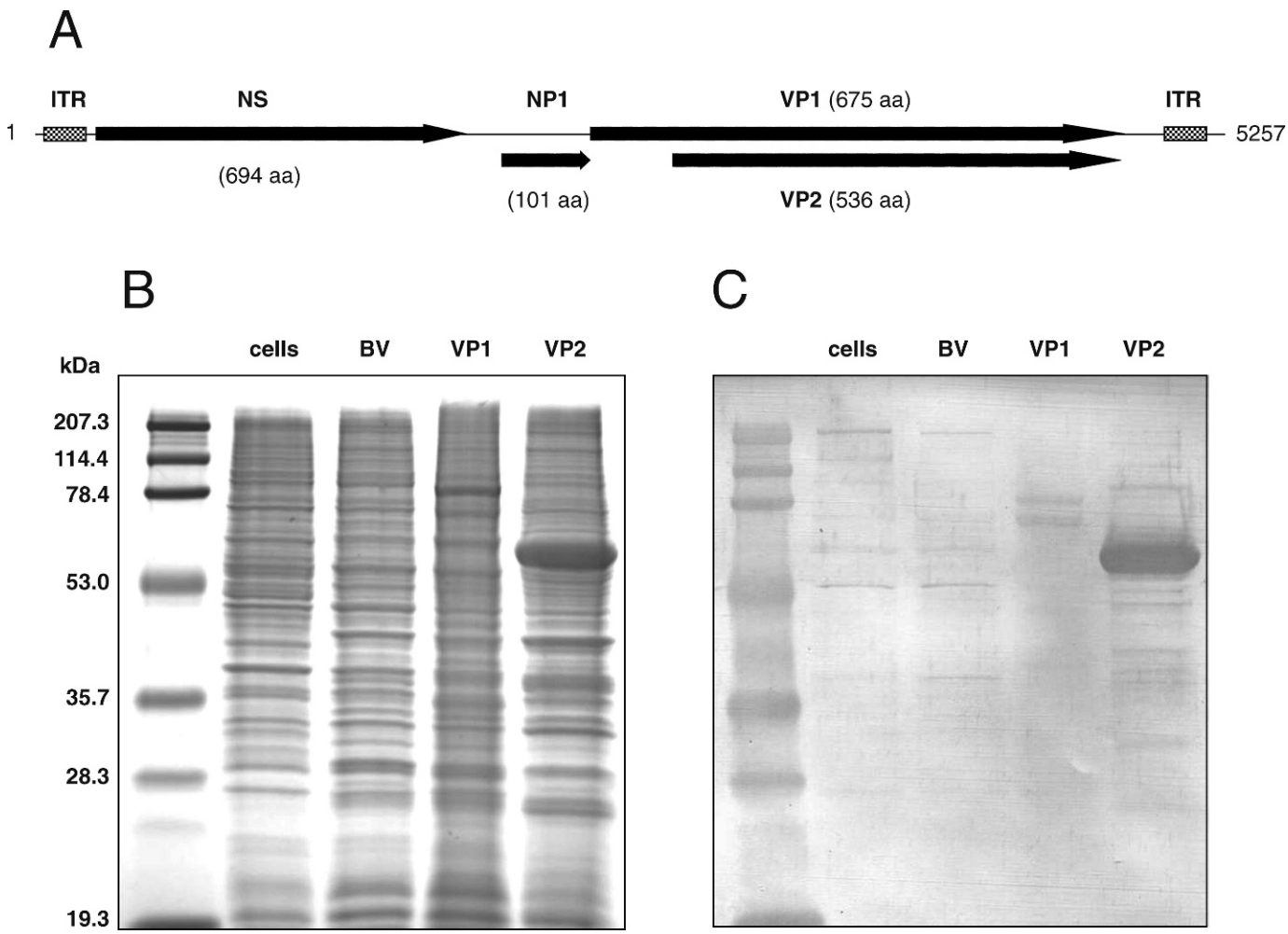

Fig. 1. Expression of ChPV-VP1 and ChPV-VP2 from baculovirus recombinants. (A) The genome structure of the chicken parvovirus (Zsak *et al.*, unpubl. data). ITR = inverted terminal repeat; NS = nonstructural gene; NP1 = nonstructural protein 1; VP = structural viral protein; aa = amino acid. (B) SDS-PAGE of expressed proteins. Cells = Sf9 cells only; BV = baculovirus without insert; VP1 = baculovirus expressing VP1; VP2 = baculovirus expressing VP2; kDa = kilodalton. (C) Western blot of expressed proteins with parvovirus-infected chicken serum.

then passaged twice more. Total DNA was extracted from virus-containing supernatants of infected SF9 cells, and confirmation of correct insertion of the VP1 and VP2 gene in the baculovirus recombinant was done by PCR amplification using baculovirus-specific primers and sequencing of the product. Further confirmation was done by performing sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel and Western blot on proteins expressed in SF9 cell culture.

For antigen production, SF9 cells were infected with ChPV-VP2 and BV-aMPV(C)-G recombinants and harvested after 72 hr. Cells were centrifuged at 500 × *g* for 10 min and washed three times in Tris-ethylenediaminetetraacetic acid (TE) buffer. Cell pellet was resuspended in 1 ml of TE buffer and subjected to three freeze-thaw cycles at −80 C. Finally, the cell lysates were sonicated and centrifuged at 5000 × *g* for 15 min, and the supernatant was used for analysis of protein production and used directly in ELISA.

ELISA. Baculovirus recombinants expressing chicken parvovirus VP2 protein (VP2Ag) and avian metapneumovirus-C subtype G protein (GAg) were used as source of antigens in ELISA. Briefly, 96-well white, flat-bottom Nunc plates (Fisher Scientific, Pittsburgh, PA) were coated with BV-VP2 infected SF9 cell lysates (48 wells on the left side of the plates) and BV-aMPV(C)-G infected SF9 cell lysates (48 wells on the right side of the plates). Coating, washing, blocking, and diluting reagents were purchased from Immunochemistry Technologies (Bloomington, MN). Five hundred nanograms of each protein in antigen coating buffer CB2 was added to the wells and incubated overnight at room temperature (RT) in light protected, humidified storage box. After three washes in washing buffer (WB1), the wells were blocked with blocking buffer 2 (BB2) blocking solution for 3 hr at RT. Next, 1:200 dilutions of chicken serum were added to the wells and incubated at RT. Negative control sera from uninfected birds (white rock, group A) were assayed in triplicate wells with both antigens in two independent plates. In subsequent assays, negative serum and test sera were added to duplicate wells on both the left and right sides of a plate coated with VP2Ag and GAg, respectively. After 1 hr, serum was removed and the wells were washed with WB1. Anti-chicken horseradish peroxidase-conjugated IgG or IgM secondary antibodies (GenWay Biotech, San Diego, CA) in 1:1000 dilution were added to each well and plates were incubated for 1 hr at RT. Bound secondary antibody was measured by chemiluminescence after a 2 min incubation with 100 µl solution of LumiGLO, a luminol-based chemiluminescent substrate (KPL, Inc., Gaithersburg, MD). Plates were then read on a Biotek FL × 800 microtiter plate luminometer (Biotek Instruments, Winooski, VT) with a 0.2 sec read time per well.

To determine the correlation between luminescence reading values and concentration of serum antibodies to VP2Ag, two different calculations were applied. First, readings in VP2Ag-coated wells were expressed as the ratio of relative light unit (RLU) obtained for test serum divided by RLU obtained for negative serum. The second calculation for each sera was based on the ratio of RLU values for VP2Ag (positive antigen) divided by RLU values for GAg (negative antigen).

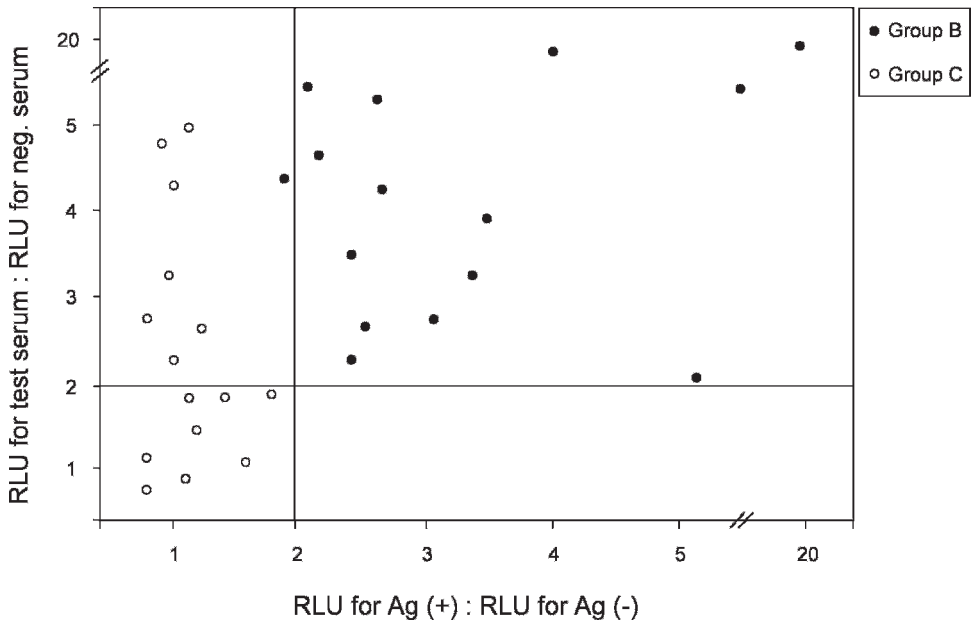

Fig. 2. Development of an ELISA to detect chicken parvovirus-specific antibodies. One group of SPF white rock chickens ($n = 15$) was infected with ChPV (group B) and the other group ($n = 15$) was kept as uninfected (group C). Sera were taken 3 wk later and tested in ELISA. On the *y* axis, readings in Ag+ wells are expressed as the ratio of RLU obtained for the test serum divided by RLU obtained for negative serum (pooled sera from group A). On the *x* axis, the ratio of RLU for VP2Ag (Ag+) divided by RLU for GAg (Ag−) is shown.

Statistics. The intra-assay variation for both the IgG and IgM ELISA was calculated by assaying three serum samples four times in two independent test plates within an assay. The interassay variation was calculated by analyzing three serum samples in five different ELISA performed on different days. The coefficient of variation (CV) was calculated as being equal to the standard deviation divided by the mean multiplied by 100 to produce a percentage (1).

RESULTS

Expression of parvovirus VP1 and VP2 proteins in baculovirus recombinants. Baculovirus recombinants containing the parvovirus VP1 and VP2 genes (ChPV-VP1 and ChPV-VP2) were transfected into SF9 cells and clones chosen for further analysis. When expressed protein from cell lysates was analyzed by SDS-PAGE gel, a prominent band of approximately 78 kDa was seen on gels for ChPV-VP1 (Fig. 1B), compared to the estimated size of 76.6 kDa, which was calculated based on amino acid composition (8). ChPV-VP2 showed a band around 60 kDa, the same size as predicted based on amino acids. The control lanes containing either SF9 cells only or baculovirus without an insert lacked the bands corresponding to VP1 and VP2. Western blots of baculovirus antigens against antisera from parvovirus-infected chickens revealed similar products for the two proteins (Fig. 1C), which were again absent from the controls.

ELISA development. Sera from five uninfected SPF chickens, which were bled at 35 days of age (white rock group A), showed similar RLU readings with VP2 antigen (Ag+) when compared to those obtained with BV-G antigen (Ag−) in ELISAs that were repeated on three independent days. Those sera were then pooled and used as negative serum in subsequent assays.

Sera from 14 of the 15 inoculated chickens (93%) exhibited an RLU ratio of more than two when the values for Ag+ wells *vs.* Ag− wells or test serum *vs.* negative serum were computed (Fig. 2). All serum samples that were collected from uninoculated birds had a less than two RLU ratio using the Ag+ wells *vs.* Ag− wells calculation. However, 7 of 15 samples (47%) in the uninoculated group showed more than two times higher readings in Ag+ wells when compared to those obtained with the negative control serum. In following assays, test sera were considered positive to parvovirus when they had an RLU ratio of two or higher as obtained by computing both the Ag+ *vs.* Ag− readings and the test *vs.* negative control sera.

To measure the reproducibility of the ELISA, coefficient of variation percentage (CV%) values were calculated. The CV% in the intra-assays with three different sera was less than 10% (Table 2). The interassay CV% revealed somewhat higher values, but each sera showed less than 15% indices when tested at five independent time points. There were no substantial differences in the CV% when the intra-assay or interassay variability was measured using either IgG or IgM ELISA.

Detection of chicken parvovirus-specific IgG and IgM antibodies. The performance of the ELISA was further evaluated in a study to detect maternally derived antibodies in 80 two-day-old fast Cornish chickens that were received from a commercial hatchery. Using the IgG ELISA, 30 of 42 (71%) serum samples from chickens that were killed at 2 days of age (group FC-A) tested positive for chicken parvovirus-specific antibodies (Fig. 3). The RLU ratio ranged between 2 and 8.9, with three samples showing values of more than 5. All of these sera were negative in IgM ELISA. The rest of the chickens ($n = 38$) were bled 3 wk later (group FC-B) and tested for antibodies. None of those birds had detectable chicken parvovirus-specific antibodies using IgG or IgM ELISA.

To determine the kinetics of antibody responses to virus infection, 42 parvovirus-infected chickens in group FC-C were bled at various times postinfection, and serum samples were assayed in IgG and IgM ELISA (Fig. 4). There were detectable IgG, but not IgM, parvovirus-specific antibodies in 70% of the birds at 5 DPI. Between 14 and 21 DPI, increasing numbers of chickens (up to 50%) showed virus-specific IgM response, and much fewer IgG positive samples could be detected at the same time points. By 32 DPI, 96% of infected chickens became seroconverted to ChPV and exhibited IgG type antibody response. Five percent of those birds also had IgM

Table 2. Coefficient of variation (CV) for both the IgG and IgM ELISA.

Assay	Serum	Intra-assay CV %					Interassay CV %
		T1[A]	T2	T3	T4	T5	
IgG ELISA	IgG (+)	8.9	7.4	6.7	7.7	5.7	13.3
	IgM (+)	4.5	4.2	6.0	6.3	5.3	10.9
	Negative	1.9	3.1	1.8	2.9	6.9	6.0
IgM ELISA	IgG (+)	4.8	1.9	4.7	3.0	5.5	11.9
	IgM (+)	1.8	2.9	4.6	2.9	6.7	12.8
	Negative	5.3	6.2	3.9	5.2	9.2	8.7

[A]T = independent days when assay was performed.

antibodies, and two chickens did not show detectable antibody response to infection.

DISCUSSION

Here, we describe the development of a capture ELISA to detect chicken parvovirus-specific antibodies in poultry. Our data indicate that the ELISA test can be successfully applied to measure antibody response following acute infection, as well as to identify maternally derived antibodies in young birds. This assay is ideally suited for use as primary serologic test to screen large numbers of samples quickly and at relatively little expense.

Earlier, we showed that chicken parvoviruses are widely distributed in commercial poultry flocks in the United States and that these viruses can potentially cause enteric diseases in broilers (35). To define the role of chicken parvoviruses in the etiology of enteric diseases of poultry, complex seroepidemiological studies are necessary. Currently, there are no reports of successful propagation of ChPV in cell cultures, and there is a lack of available tests to use in serologic assays to detect virus-specific antibodies.

In our study, we used an approach previously applied to express parvovirus structural proteins in insect cells from baculovirus recombinants (13,15,18,20). In those studies, baculovirus recombinant-expressed VP2 antigen worked well in ELISAs, which were employed for the detection of antibodies to human B19 parvovirus, boca parvoviruses, and mouse parvovirus infections (13,19,20,30). Similarly, our results demonstrate that recombinant ChPV-VP2 antigen can be used in detection assays for immunoglobulins directed against viral structural determinants.

In baculovirus recombinant-infected cells, ChPV-VP2 protein was detected as a much more abundant protein than the minor structural VP1 protein. Importantly, the 60-kDa VP2 gave positive reaction with parvovirus-infected chicken serum in Western blot, confirming its immunoreactivity to virus-specific antibodies. This is consistent with other reports that showed that the VP2 protein is the major capsid protein of the parvovirus virion and is presented to the immune system upon exposure to the virus or during viral replication (12,15,20).

In our assay, a semipurified VP2 was used as antigen in ELISA. Other works using baculovirus recombinant-expressed parvovirus VP2 antigen showed that similarly prepared, semipurified VP2 provided high sensitivity and specificity to detect antibodies in ELISA tests (20). Although reports indicate that highly purified VP2 antigen can improve the sensitivity of ELISA tests for detection of antibodies to human parvoviruses (13), this proved to be unnecessary because the semipurified ChPV-VP2 preparation was strongly immunoreactive and therefore required no further purification.

We have used sera from experimentally infected chickens and sera from uninfected birds to evaluate the performance of the ELISA. The assay performed with high specificity and sensitivity, where 100% of uninfected chicken serum samples showed negative results and 93.3% of infected chicken sera were positive in our test. The high specificity and reproducibility of the assay eliminate the need for developing additional serologic tests for detection of anti-ChPV antibodies.

The ChPV-VP2 ELISA was used to detect parvovirus-specific antibodies in young chickens. Interestingly, a high percentage (71.4%) of day-old chickens contained detectable IgG (but not IgM) type serum antibodies, indicating that their parents had been previously infected with chicken parvovirus and virus-specific immunoglobulins were transferred as maternal antibodies to their

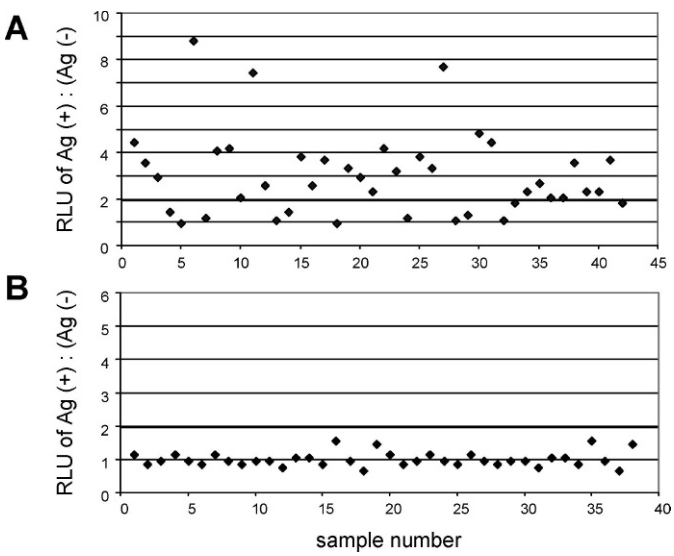

Fig. 3. Detection of maternally acquired antibodies to chicken parvovirus. Eighty 2-day-old broiler chickens were received from a commercial hatchery. (A) Serum was collected from 42 chickens immediately and (B) the remaining 38 chickens were bled after 3 wk. Sera were tested using the ChPV-VP2 ELISA. Readings are expressed as the ratio of RLU obtained for positive antigen (Ag+) divided by RLU values for negative antigen (Ag−).

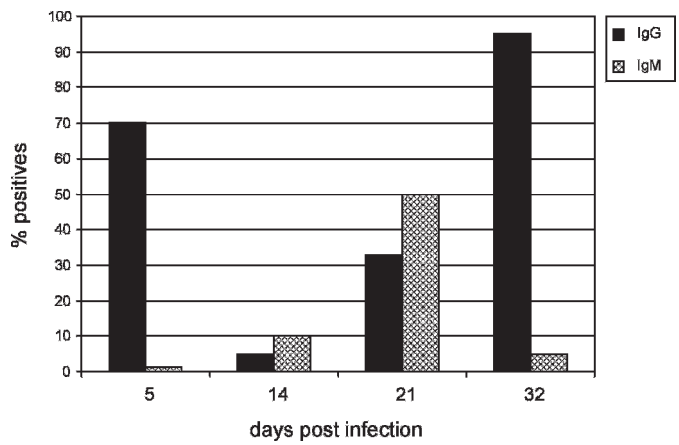

Fig. 4. Antibody production following chicken parvovirus infection. Commercial broiler chickens at 2 days of age ($n = 42$) were inoculated orally with ABU-P1 chicken parvovirus. Serum samples were collected from each bird at 5, 14, 21, and 32 DPI and ChPV-VP2 ELISA was performed as described in the Materials and Methods section.

progenies. As expected, these maternal antibodies were cleared in 3 wk, and without acute virus exposure the chickens became seronegative to ChPV.

This is the first time that maternally derived antibodies to chicken parvovirus have been described in young birds. The data further support our previous findings that parvovirus infection is highly prevalent in poultry (35) and show that a specific immunoglobulin response can persist for a long time following ChPV infection. Notably, maternally acquired virus-specific antibodies have an important role in the epidemiology of many parvovirus infections, including the goose parvovirus-induced Derzsy's disease, and the level of maternal antibodies plays a significant role in protection against disease (10). It is reasonable to speculate that the presence of ChPV-specific antibodies in chickens also determines the susceptibility of the birds to parvovirus infection. Our current ongoing studies are focused to confirm whether this is indeed the case.

The ChPV-VP2 ELISA performed well when it was tested to detect humoral antibody response following virus infection. In general, the pattern of parvovirus replication and antibody production is similar among the various parvovirus species (4). While maternally derived antibodies are IgG type immunoglobulins, virus infection is followed by production of specific IgM, and some time later specific IgG antibodies are produced. In our study, a typical immune response of the chickens was observed starting with IgM production between 14 DPI and 21 DPI and switching into a predominant IgG immune response by 32 DPI. Differentiating between IgG and IgM type antibody responses and the ability to distinguish between maternally acquired antibodies and antibodies produced following acute infection could prove to be a useful tool in future experiments to characterize pathobiological properties and immunogenicity of ChPV.

REFERENCES

1. Banerjee, M., S. Bhattacharyya, and C. Das. Development of a highly sensitive direct microtiter plate ELISA for human serum testosterone. Indian J. Clin. Biochem. 14:19–25. 1999.

2. Barnes, H. J., and J. S. Guy. Poult enteritis-mortality syndrome. In: Diseases of poultry, 11th ed. Y. M. Saif, ed. Blackwell Publishing, Ames, IA. pp. 1171–1180. 2003.

3. Barnes, H. J., J. S. Guy, and J. P. Vaillancourt. Poult enteritis complex. Rev. Sci. Technol. 19:565–588. 2000.

4. Bloom, M. E., and J. R. Kerr. Pathogenesis of parvovirus infections. In: Parvovirus. J. R. Kerr, S. F. Cotmore, M. E. Bloom, R. M. Linden, and C. R. Parish, eds. Hodder Arnold, London. pp. 323–341. 2006.

5. Craig, J. V., W. F. Dean, G. B. Havenstein, K. K. Kruger, K. E. Nestor, G. H. Purchase, P. B. Siegel, and G. L. van Wicklen. Guidelines for poultry husbandry. In: Guide for the care and use of agricultural animals in agricultural research and teaching. Federation of American Science Societies, Savoy, IL. pp. 55–66. 1999.

6. Day, J. M., M. J. Pantin-Jackwood, and E. Spackman. Sequence and phylogenetic analysis of the S1 genome segment of turkey-origin reoviruses. Virus Genes 35:235–242. 2007.

7. Day, J. M., E. Spackman, and M. Pantin-Jackwood. A multiplex RT-PCR test for the differential identification of turkey astrovirus type 1, turkey astrovirus type 2, chicken astrovirus, avian nephritis virus, and avian rotavirus. Avian Dis. 51:681–684. 2007.

8. Gasteiger, E., C. Hoogland, A. Gattiker, S. Duvaud, M. R. Wilkins, R. D. Appel, and A. Bairoch. Protein identification and analysis tools on the ExPASy server. In: The proteomics protocols handbook. J. M. Walker, ed. Humana Press, Totowa, NJ. pp. 571–607. 2005.

9. Goodwin, M. A., J. F. Davis, M. S. McNulty, J. Brown, and E. C. Player. Enteritis (so-called runting stunting syndrome) in Georgia broiler chicks. Avian Dis. 37:451–458. 1993.

10. Gough, R. E. Goose parvovirus infection. In: Diseases of poultry, 11th ed. Y. M. Saif, ed. Blackwell Publishing, Ames, IA. pp. 367–374. 2003.

11. Guy, J. S. Virus infections of the gastrointestinal tract of poultry. Poult. Sci. 77:1166–1175. 1998.

12. Hedman, K., R. Franssila, L. Kaikkonen, K. Hokynar, L. Kakkola, and M. Soderlund-Venermo. Immunology of human erythrovirus infection. In: Parvoviruses. J. R. Kerr, S. F. Cotmore, M. E. Bloom, R. M. Linden, and C. R. Parish, eds. Hodder Arnold, London. pp. 351–363. 2006.

13. Kahn, J. S., D. Kesebir, S. F. Cotmore, A. D'Abramo, Jr., C. Cosby, C. Weibel, and P. Tattersall. Seroepidemiology of human bocavirus defined using recombinant virus-like particles. J. Infect. Dis. 198:41–50. 2008.

14. Kisary, J. Experimental infection of chicken embryos and day-old chickens with parvovirus of chicken origin. Avian Pathol. 14:1–7. 1985.

15. Kisary, J. RecombiVac-S, the first Hungarian biosynthetic (recombinant) subunit vaccine. Magyar Allatorvosok Lapja 121:243–247. 1999.

16. Kisary, J., B. Nagy, and Z. Bitay. Presence of parvoviruses in the intestine of chickens showing stunting syndrome. Avian Pathol. 13:339–343. 1984.

17. Koci, M. D., B. S. Seal, and S. Schultz-Cherry. Molecular characterization of an avian astrovirus. J. Virol. 74:6173–6177. 2000.

18. Le Gall-Recule, G., V. Jestin, P. Chagnaud, P. Blanchard, and A. Jestin. Expression of muscovy duck parvovirus capsid proteins (VP2 and VP3) in a baculovirus expression system and demonstration of immunity induced by the recombinant proteins. J. Gen. Virol. 77:2159–2163. 1996.

19. Lin, F., W. Guan, F. Cheng, N. Yang, D. Pintel, and J. Qiu. ELISAs using human bocavirus VP2 virus-like particles for detection of antibodies against HBoV. J. Virol. Methods 149:110–117. 2008.

20. Livingston, R. S., D. G. Besselsen, E. K. Steffen, C. L. Besch-Williford, C. L. Franklin, and L. K. Riley. Serodiagnosis of mice minute virus and mouse parvovirus infections in mice by enzyme-linked immunosorbent assay with baculovirus-expressed recombinant VP2 proteins. Clin. Diagn. Lab. Immunol. 9:1025–1031. 2002.

21. Lwamba, H. C., R. Alvarez, M. G. Wise, Q. Yu, D. Halvorson, M. K. Njenga, and B. S. Seal. Comparison of the full-length genome sequence of avian metapneumovirus subtype C with other paramyxoviruses. Virus Res. 107:83–92. 2005.

22. Page, R. K., O. J. Fletcher, G. N. Rowland, D. Gaudry, and P. Villegas. Malabsorption syndrome in broiler chickens. Avian Dis. 26:618–624. 1982.

23. Pantin-Jackwood, M. J., J. M. Day, M. W. Jackwood, and E. Spackman. Enteric viruses detected by molecular methods in commercial chicken and turkey flocks in the United States between 2005 and 2006. Avian Dis. 52:235–244. 2008.

24. Pantin-Jackwood, M. J., E. Spackman, and J. M. Day. Pathology and virus tissue distribution of turkey origin reoviruses in experimentally infected turkey poults. Vet. Pathol. 44:185–195. 2007.

25. Pantin-Jackwood, M. J., E. Spackman, and J. M. Day. Pathogenesis of type 2 turkey astroviruses with variant capsid genes in 2-day-old specific pathogen free poults. Avian Pathol. 37:193–201. 2008.

26. Pantin-Jackwood, M. J., E. Spackman, J. M. Day, and D. Rives. Periodic monitoring of commercial turkeys for enteric viruses indicates continuous presence of astrovirus and rotavirus on the farms. Avian Dis. 51:674–680. 2007.

27. Pantin-Jackwood, M. J., E. Spackman, and P. R. Woolcock. Phylogenetic analysis of turkey astroviruses reveals evidence of recombination. Virus Genes 32:187–192. 2006.

28. Pass, D. A., M. D. Robertson, and G. E. Wilcox. Runting syndrome in broiler chickens in Australia. Vet. Rec. 110:386–387. 1982.

29. Reynolds, D. L., Y. M. Saif, and K. W. Theil. Enteric viral infections of turkey poults: incidence of infection. Avian Dis. 31:272–276. 1987.

30. Salimans, M. M., M. J. van Bussel, C. S. Brown, and W. J. Spaan. Recombinant parvovirus B19 capsids as a new substrate for detection of B19-specific IgG and IgM antibodies by an enzyme-linked immunosorbent assay. J. Virol. Methods 39:247–258. 1992.

31. Spackman, E., D. Kapczynski, and H. Sellers. Multiplex real-time reverse transcription-polymerase chain reaction for the detection of three viruses associated with poult enteritis complex: turkey astrovirus, turkey coronavirus, and turkey reovirus. Avian Dis. 49:86–91. 2005.

32. Spackman, E., M. Pantin-Jackwood, J. M. Day, and H. Sellers. The pathogenesis of turkey origin reoviruses in turkeys and chickens. Avian Pathol. 34:291–296. 2005.

33. Trampel, D. W., D. A. Kinden, R. F. Solorzano, and P. L. Stogsdill. Parvovirus-like enteropathy in Missouri turkeys. Avian Dis. 27:49–54. 1983.

34. Yu, M., M. M. Ismail, M. A. Qureshi, R. N. Dearth, H. J. Barnes, and Y. M. Saif. Viral agents associated with poult enteritis and mortality syndrome: the role of a small round virus and a turkey coronavirus. Avian Dis. 44:297–304. 2000.

35. Zsak, L., K. O. Strother, and J. M. Day. Development of a polymerase chain reaction procedure for detection of chicken and turkey parvoviruses. Avian Dis. 53:83–88. 2009.

36. Zsak, L., K. O. Strother, and J. Kisary. Partial genome sequence analysis of parvoviruses associated with enteric disease in poultry. Avian Pathol. 37:435–441. 2008.

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